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Associations between urinary phthalate concentrations and semen quality parameters in a general population

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STUDY QUESTION: Are urinary phthalate concentrations associated with altered semen quality parameters among males recruited from the general population?

SUMMARY ANSWER: Urinary levels of metabolites of phthalate diesters are associated with lower total sperm counts, larger sperm head sizes, and higher percentages of morphologically abnormal sperm.

WHAT IS KNOWN ALREADY: High dose experiments in rats implicate phthalates as anti-androgens. Studies involving infertile men seeking care suggest that phthalates influence measures of semen quality raising concern about the implications for men in the general population.

STUDY DESIGN, SIZE, DURATION: This prospective cohort study comprised 501 male partners in couples discontinuing contraception to become pregnant, who were recruited from 16 US counties using population-based sampling frameworks from 2005 to 2009.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Urine and semen samples were obtained at baseline from 473 (94%) men, of whom 378 (80%) men provided a second sample the following month. Urine was analyzed for 14 monoester metabolites of phthalate diesters by high-performance liquid chromatography coupled to tandem mass spectrometry. Semen samples were analyzed for 34 quality parameters categorized as general, motility, morphology, sperm head and sperm chromatin structure.

MAIN RESULTS AND THE ROLE OF CHANCE: Urinary mono-[2-(carboxymethyl) hexyl] phthalate (MCMHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-benzyl phthalate (MBzP), and mono-isononyl phthalate (MNP) were significantly associated with lower total sperm counts and concentrations, larger sperm head sizes, higher proportions of megalo head sperm morphology, and/or other morphological changes. Urinary mono-methyl phthalate (MMP) and mono-cyclohexyl phthalate (MCPP) were significantly associated with lower sperm motility, and urine mono-2-ethylhexyl phthalate (MEHP) was significantly associated with higher sperm motility.

LIMITATIONS, REASONS FOR CAUTION: While adverse associations were observed, the implications of the findings for couple fecundity and fertility remain to be established. Cautious interpretation is needed in light of reliance on a single measurement of phthalate measure and no correction for multiple comparisons.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (N01-HD-3-3355, N01-HD-3-3356 and NOH-HD-3-3358). The authors declare they have no actual or potential competing financial interests.

Key words: endocrine disrupting chemicals / phthalates / plasticizers / semen / sperm

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Introduction

Phthalate diesters are used broadly in consumer products, leading to widespread human exposure and concern about potential implications for human health. These organic compounds have long been used as plasticizers in a myriad of commercial applications (Wittassek et al., 2011; Guo and Kannan, 2013). High molecular weight phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DNP), are incorporated into applications that utilize flexible vinyl plastics, whereas low molecular weight phthalates, such as dibutyl phthalate (DBP) and diethyl phthalate (DEP), are used as solvents and coatings (Hauser and Calafat, 2005; Guo and Kannan, 2013). Once absorbed, phthalate diesters are quickly metabolized into monoesters, which are biologically active and ultimately excreted in urine where they can be measured. Phthalates are considered to be ubiquitous in the modern environment, and human exposure frequently occurs through ingestion of foods contaminated by phthalates used in packaging, coatings used in medications, and solvents used in personal care products (Koch et al., 2013). Human biomonitoring data suggest that measurable levels of phthalate are common in the general US population (CDC, 2015).

A growing body of evidence, animal and human, suggests effects of phthalates on male reproductive function (Foster, 2006; Kay et al., 2014). Male rodents exposed to non-toxic doses of DEHP and DBP *in utero*, and during the early post-natal period, have displayed anti-androgenic effects (Foster et al., 2001; Miura et al., 2007) and reduced testosterone production (Parks et al., 2000), although these effects have not been observed uniformly. Conversely, marmosets with fetal and neonatal exposure to DEHP and monobutyl phthalate (MBP) have displayed neither short-term nor long-term effects on hormone production or reproductive physiology (Tomonari et al., 2006; McKinnell et al., 2009). Results from *in vitro* testicular tissue explant studies are likewise discordant; impaired steroidogenesis and severe germ cell dysfunction have been reported for rat specimens, but not for human specimens treated with DBP (Heger et al., 2012; Mitchell et al., 2012), although testosterone synthesis was decreased by DEHP (Desdoits-Lethimonier et al., 2012). The results from human epidemiologic studies of sex-steroid hormones have also been inconsistent (Meeker et al., 2009; Meeker and Ferguson, 2014).

Data in human populations are limited with respect to phthalates and male reproductive health, and largely rely on men/couples seeking infertility evaluation or treatment (Kay et al., 2014). Such findings include MBP being associated with lower sperm concentration and poorer morphology, and monoethyl phthalate (MEP) being associated with sperm DNA damage, and similarly so, for mono-(2-ethylhexyl) phthalate (MEHP) after adjustment for other DEHP metabolites (Hauser et al., 2007). However, no such associations have been observed in studies evaluating phthalates and semen quality among non-clinical samples in Sweden (Jönsson et al., 2005), Denmark (Joensen et al., 2012) and China (Han et al., 2014), raising questions about the generalizability of findings from clinical settings to populations. Another limitation of past research is the reliance on a few semen quality end-points such as sperm concentration, count and morphology (Kay et al., 2014), which are reported to have limited clinical value in the absence of extremes (Lewis, 2007). Other challenges include the wide spectrum of biologically active primary and secondary metabolites of phthalates, with apparently heterogeneous effects (Hauser and Calafat, 2005).

Thus, the objective of our study was to evaluate the impact of phthalate diester exposure on male reproductive health using a non-clinical sample of males and a broad panel of measures of semen quality. We sought to explore the relation between phthalate concentrations and a diverse array of semen quality end-points in men recruited from the general population.

Materials and Methods

Study sample

The current analysis was performed using data from male participants in the Longitudinal Study of Infertility and the Environment (LIFE), a prospective cohort study that enrolled couples discontinuing contraception to conceive a pregnancy, the details of which have been previously published (Buck Louis et al., 2011). In brief, a total of 501 couples were recruited from 16 counties at study sites in Michigan and Texas from 2005 to 2009; 42% of couples who completed screening and were eligible to participate enrolled in the study. Because of the absence of uniform registries of couples planning pregnancy to use as a sampling frame (Buck et al., 2004), the Texas fishing/hunting license registry and a commercial marketing database in Michigan were used to identify potential participants. Eligible men were aged at least 18 years, in a committed relationship, and capable of communication in English or Spanish. Men with physician-diagnosed infertility were ineligible for participation.

We conducted baseline interviews in participants' homes at the time of enrollment, took anthropometric measurements, and collected biologic specimens. Nurse interviewers collected information regarding demographics, health and reproductive histories, physical activity, and medication/supplement use. Non-fasting blood and spot urine specimens were collected at the time of the baseline interview, and the specimens were stored on ice until transportation to study site laboratories for storage at −80°C.

The study protocol entailed collection of two semen samples: one at baseline and another after one month, as has been described in detail previously (Buck Louis et al., 2015). Home collection kits were provided, which included insulated shipping containers, shipping materials, cold packs, and glass specimen jars with an attached thermometer. The men were instructed to collect semen samples by masturbation following two days of abstinence and without use of lubricants. Men recorded the date and time of collection on the label of the specimen, noted if any sample was spilled (yes/no), and noted the length of abstinence or time since last ejaculation in days. Semen was shipped on cold packs overnight, arriving at the andrology laboratory for analysis the next day.

Semen quality assessment

Thirty-four semen quality parameters were assessed by the National Institute of Occupational Safety and Health (NIOSH) Andrology Laboratory (Cincinnati, OH, USA). Upon arrival at the lab, semen specimens were checked to verify temperature between −0.5 and 15°C, and then warmed to 37°C. Volume was measured to the nearest 0.1 ml and viability was determined using the hypo-osmotic swelling (HOS) assay (Jeyendran et al., 1992). A semen aliquot was placed into a 20-μm-depth micro chamber slide (Leja, Nieuw-Vennep, Netherlands) and eight measures of motility were determined using the HTM-IVOS computer aided semen analysis (CASA) platform with video playback (Hamilton Thorne Biosciences, Beverly, MA, USA). Azoospermia denoted the absence of sperm in both samples, and such men were informed and advised to seek clinical care.

For each semen specimen, four standard microscopic slides were prepared with IDENT™ stain by Fertility Solutions® (Cleveland, OH, USA). IDENT™ slides were used to determine sperm total count and concentration using the HTM-IVOS CASA platform (Hamilton Thorne Biosciences),

and also to assess 14 measures of sperm morphology and 6 measures of sperm head morphometry (Schrader *et al.*, 1990) using the IVOS METRIX system (Hamilton Thorne Biosciences). For overall sperm morphology, we applied both the third edition of the World Health Organization (WHO) morphologic criteria (i.e. 'traditional' criteria) and Kruger's Tygerberg criteria (i.e. 'strict' criteria). The traditional criteria stipulates a minimum 30% normal forms, using parameters based on multinational observational study data, and are more liberal than the strict criteria (World Health Organization, 1992; Rothmann *et al.*, 2013). The strict criteria stipulates a minimum of 4% normal forms; however, the definition of 'normal' is more conservative than the traditional criteria, using parameters based on post coital *in vivo* data (Kruger *et al.*, 1988; Menkveld and Kruger, 1995).

The sperm chromatin structure assay (SCSA®) for DNA stability was conducted using a Coulter Epics Elite Flow Cytometer (SCSA Diagnostics, Brookings, SD, USA) following dilution of whole semen in TNE buffer (Evenson, 2013). SCSA® measures DNA damage, which is then quantified as the DNA fragmentation index (DFI), describing the percentage of damaged DNA, and as % high stainability, describing the percentage of immature sperm nuclei with abnormal proteins (Evenson, 2013).

All semen analyses were conducted according to NIOSH Standard Operating Procedures quality assurance guidelines and in compliance with American Society of Andrology quality control parameters (American Society of Andrology, 1996). Data were inspected and Westgard rules (Westgard *et al.*, 1981) implemented to ensure the absence of batch related differences and laboratory drift. Complete analysis was conducted on the first semen specimen and an abridged analysis was conducted on the second semen specimen, assessing only volume, viability, total count, concentration, motility, and sperm head morphology.

Urine phthalate assessment

Urine and blood specimens were shipped on dry ice to the Wadsworth Center, New York State Department of Health (Albany, NY, USA) for laboratory analysis. Phthalate monoester metabolites in urine were determined using high-performance liquid chromatography with electrospray triple-quadrupole tandem mass spectrometry (HPLC-MS/MS) according to previously described methods (Guo *et al.*, 2011; Buck Louis *et al.*, 2014). We determined 14 phthalate monoesters (ng/ml), including: MEHP, mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), mono (2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-methyl phthalate (MMP), MEP, mono (3-carboxypropyl) phthalate (MCPP), monooctyl phthalate (MOP), mono-isononyl phthalate (MNP), mono (2-isobutyl) phthalate (MiBP), MBP, monocyclohexyl phthalate (MCHP), and monobenzyl phthalate (MBzP). Limits of quantification (LOQs) were determined as twice the value of the lowest valid calibration standard concentration, and ranged from 0.05–1.0 ng/ml. We quantified urine creatinine with a commercial kit using a Roche Hitachi 912 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN, USA). Serum cotinine was determined in ng/ml by HPLC-MS/MS (Bernert *et al.*, 1997).

Statistical analysis

We characterized the distributions of phthalate metabolites, semen parameters, and covariates, and identified outliers for further examination. We used all machine-observed phthalate concentrations without imputation of values below LOQs (Schisterman *et al.*, 2006). We evaluated bivariate associations among phthalates, adjusting for urine creatinine as a covariate in the model. In separate general linear models, we assessed associations between semen parameters as outcomes, and individual phthalates adjusted for a set of covariates identified a priori from the existing literature, including urine creatinine in mg/dl, age in years (Johnson *et al.*, 2015), body mass index in kg/m^2 (Hatch *et al.*, 2008; Eisenberg *et al.*, 2014), active smoking (yes, defined as serum cotinine >10 ng/ml versus no) (Ramlau-Hansen *et al.*, 2007; Benowitz *et al.*, 2009), race (white versus others), income ($50 000–$89 999 versus < $49 999 and ≥ $90 000 versus < $49 999), and study site (Michigan versus Texas), for observations with complete information ($n = 375$). We included abstinence time as a covariate (days) to improve model fit; it was not a confounder but is a strong predictor of semen quality (Carlsen *et al.*, 2004). We also constructed general linear mixed models to accommodate the use of up to 2 semen samples per man for associations between phthalates and semen volume, viability, total sperm count, sperm concentration, motility, and sperm head morphology; 378 of 473 participants (80%) provided 2 semen samples. We also looked at the additive effect of phthalates using $\sum$DEHPm, the molar sum of MEHP, MCMHP, MEHHP, MEOHP, and MECPP.

Phthalates were natural log-transformed and rescaled to units of one interquartile range (IQR) to aid interpretation; regression coefficient estimates correspond to the difference in the dependent variable associated with each one IQR change in individual phthalate concentrations. We used Box-Cox transformations to the power 'k' (Supplementary Table SI) defined as $Y' = (Y^k - 1)/k$ for $k > 0$ and $Y' = \log(Y)$ for $k = 0$, to achieve normality of semen quality parameters assessed by Shapiro Wilk W statistics (Handelsman, 2002). We confirmed normality assumptions by examining residual plots. Azoospermic men ($n = 5$) were included only for the analysis of volume, total count, and sperm concentration. Consistent with the exploratory nature of our study, we did not adjust for type-I error inflation due to multiple comparisons, consistent with our intent to identify possible signals for future inquiry (Savitz and Olshan, 1995). *P*-values <0.05 for two-tailed tests were considered statistically significant.

Ethical approval

Study participants gave informed consent before any data collection and human subjects approval was received from all collaborating institutions.

Results

Demographic and study characteristics of participants are shown in Table I. Men were 31.8 years of age on average (range 19–51) and mostly white (78.6%), with 59% earning at least $90 000 per year. A majority of the participants were recruited from the Texas site, and few were active smokers ($n = 98$), as indicated by serum cotinine >10 ng/ml. Most (98.7%) men had abstained from ejaculation for at least two days prior to semen collection (median 3.0 days). There were $n = 5$ azoospermic men, while $n = 7$ had cryptorchidism ($n = 4$ surgically corrected), $n = 2$ had surgically corrected hypospadias, and $n = 8$ had varicocele ($n = 2$ surgically corrected).

Results of the urinary phthalates analysis are shown in Table II. Negative concentrations reflect the blank correction process used in quantification. Of the 14 monoesters, 9 were quantified above the LOQ in >95% of participants: MCMHP, MEHHP, MEOHP, MECPP, MEP, MCPP, MiBP, MBP and MBzP. Levels of MOP, MNP and MCHP were above quantification limits in ≤5% of the study sample. MEP had the highest median concentration overall (86.4 ng/ml), followed by MECPP (20.4 ng/ml), MCMHP (18.5 ng/ml) and MEHHP (15.2 ng/ml). As shown in Supplementary Table SII, we detected a range of moderate to strong creatinine-adjusted correlations among the DEHP metabolites MEHP, MCMHP, MEHHP, MEOHP and MECPP ($r = 0.44–0.94$, $P < 0.001$), while the DBP metabolites MBP and MiBP were moderately correlated ($r = 0.51$, $P < 0.001$).

Results for the regression analysis of the relations of continuous phthalate metabolites with semen parameters, adjusted for age, cotinine,

Table I Demographic and study characteristics of male LIFE Study participants ($n = 473$).

Factors	*n* (%)	Mean	SD	Median	Minimum	Maximum
Age (year)	473	31.8	4.9	31.0	19.0	51.0
BMI (kg/m^2)	468	29.9	5.6	28.9	18.1	57.8
Race						
White	371 (78.6)	–	–	–	–	–
Other	101 (21.4)	–	–	–	–	–
Income ($)						
<10 000–49 999	71 (15.2)	–	–	–	–	–
50 000–89 999	120 (25.8)	–	–	–	–	–
≥90 000	275 (59.0)	–	–	–	–	–
Study site						
Michigan	98 (20.7)	–	–	–	–	–
Texas	375 (79.3)	–	–	–	–	–
Serum cotinine (ng/ml)[a]	466	54.9	136	0.04	0	926
Active smoker	98 (21.0)	–	–	–	–	–
Non-smoker	368 (79.0)	–	–	–	–	–
Abstinence time (days)	469	4.1	3.7	3.0	1.0	46.5
Urine creatinine (mg/dl)	375	142.1	84.8	139.1	13.9	434.1

[a]Active smoker, cotinine >10 ng/ml; Non-smoker, cotinine ≤10 ng/ml.

Table II Distribution of phthalate monoesters (ng/ml urine) measured in male LIFE Study participants ($n = 473$).

Analyte	LOQ	% < LOQ	Minimum	Percentile			Maximum
				25th	Median	75th	
MEHP	1.0	48.6	−10.6	−1.57	1.18	4.87	733
MCMHP	0.2	0.2	−0.47	6.61	18.5	47.4	803
MEHHP	0.2	1.0	0.01	5.56	15.2	37.94	3116
MEOHP	0.2	1.2	0.01	3.06	6.95	17.9	914
MECPP	0.2	0.7	−0.05	8.60	20.4	46.4	3308
MMP	1.0	61.7	−0.48	0.02	0.54	1.98	72
MEP	0.2	0.2	0.08	31.73	86.4	270	6733
MCPP	0.2	2.9	−0.29	2.46	5.56	11.9	1089
MOP	0.5	96.4	−0.32	−0.12	−0.05	0.02	7.45
MNP	0.5	95.5	−0.62	−0.07	0	0.08	43.8
MiBP	0.2	2.4	−0.58	1.81	4.36	9.04	77.4
MBP	0.2	0.7	−0.02	3.30	7.28	14.8	2708
MCHP	0.2	95.9	−0.17	−0.01	0.00	0.01	6.82
MBzP	0.2	3.8	−0.10	1.56	3.57	8.50	420

Note: Negative concentrations reflect the blank correction process used in quantification.
LOQ, limit of quantification; MEHP, mono-(2-ethylhexyl phthalate); MCMHP, mono-[(2-carboxymethyl) hexyl] phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MMP, mono-methyl phthalate; MEP, mono-ethyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MOP, mono-n-octyl phthalate; MNP, mono-isononyl phthalate; MiBP, mono-(2-isobutyl phthalate); MBP, mono-*n*-butyl phthalate; MCHP, mono-cylcohexyl phthalate; MBzP, monobenzyl phthalate.

and other covariates are shown in Tables III–V and summarized graphically in Table VI; we presented the quantitative results for the 14 phthalate monoesters in three tables according to parent diester molecules for clarity. Multiple phthalates were associated with lower total sperm count, including higher concentrations of MCMHP (-2.89×10^6), MEHHP (-2.85×10^6), MNP (-7.20×10^6) and MBzP (-4.96×10^6). Similarly, higher levels of MCMHP (-2.20×10^6/ml), MEHHP (-1.92×10^6/ml), MEOHP (-1.90×10^6/ml), MMP (-2.11×10^6/ml), MNP

Table III Differences in semen quality parameters associated with a one interquartile range increase in natural log-transformed urine di-ethyl hexyl phthalate (DEHP) metabolites—LIFE Study (n = 375).

Semen parameter	MEHP		MCMHP		MEHHP		MEOHP		MECPP	
	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
General characteristics[a]										
Volume (ml)	0.34	(−0.31, 1.00)	0.05	(−0.40, 0.49)	−0.06	(−0.51, 0.39)	0.07	(−0.35, 0.49)	0.10	(−0.38, 0.58)
Viability (%)	4.94	(−4.13, 14.01)	−2.86	(−9.04, 3.32)	−1.33	(−7.52, 4.87)	−1.36	(−7.16, 4.45)	−0.70	(−7.38, 5.98)
Total count ($\times 10^6$)	0.17	(−3.88, 4.22)	**−2.89**	**(−5.62, −0.17)***	**−2.85**	**(−5.59, −0.11)***	−2.43	(−5.00, 0.13)	−2.29	(−5.26, 0.67)
Sperm concentration ($\times 10^6$/ml)	−0.69	(−3.44, 2.07)	**−2.20**	**(−4.05, −0.35)***	**−1.92**	**(−3.78, −0.05)***	**−1.90**	**(−3.65, −0.16)***	−1.89	(−3.91, 0.12)
Sperm motility[a]										
Average path velocity (μm/s)	**11.61**	**(0.70, 22.51)***	0.09	(−7.37, 7.56)	0.31	(−7.17, 7.79)	−2.05	(−9.05, 4.96)	−0.99	(−9.06, 7.08)
Straight line velocity (μm/s)	7.80	(−1.19, 16.78)	−1.58	(−7.72, 4.55)	−0.83	(−6.98, 5.32)	−2.96	(−8.71, 2.80)	−2.52	(−9.15, 4.11)
Curvilinear velocity (μm/s)	**20.36**	**(1.32, 39.4)***	4.01	(−9.02, 17.03)	4.88	(−8.18, 17.94)	0.69	(−11.55, 12.93)	2.27	(−11.81, 16.36)
Amplitude head displacement (μm)	**1.72**	**(0.53, 2.92)****	0.63	(−0.19, 1.45)	0.55	(−0.27, 1.37)	0.52	(−0.25, 1.29)	0.55	(−0.34, 1.43)
Beat cross frequency (Hz)	5.11	(−0.92, 11.14)	0.01	(−4.11, 4.14)	0.31	(−3.83, 4.44)	0.63	(−3.25, 4.50)	0.86	(−3.59, 5.31)
Straightness (%)	6.75	(−9.88, 23.38)	−3.44	(−14.77, 7.90)	−5.07	(−16.41, 6.27)	−5.95	(−16.57, 4.67)	−6.45	(−18.66, 5.77)
Linearity (%)	1.27	(−9.52, 12.07)	−4.61	(−11.97, 2.74)	−6.06	(−13.40, 1.28)	−6.57	(−13.44, 0.30)	−6.82	(−14.73, 1.08)
Percent motility (%)	0.93	(−1.60, 3.46)	**−2.01**	**(−3.72, −0.31)***	−1.46	(−3.18, 0.26)	**−1.61**	**(−3.22, 0.00)***	**−1.88**	**(−3.73, −0.03)***
Sperm head[a]										
Length (μm)	0.01	(−0.04, 0.07)	**0.04**	**(0.00, 0.08)***	**0.04**	**(0.00, 0.08)***	0.03	(−0.01, 0.06)	0.04	(0.00, 0.08)
Area (μm^2)	0.42	(−0.39, 1.22)	0.37	(−0.18, 0.91)	0.53	(−0.01, 1.08)	0.36	(−0.15, 0.87)	**0.72**	**(0.14, 1.31)***
Width (μm)	0.06	(−0.10, 0.23)	−0.01	(−0.12, 0.10)	0.02	(−0.09, 0.14)	0.01	(−0.09, 0.12)	0.08	(−0.04, 0.20)
Perimeter (μm)	0.21	(−0.25, 0.68)	**0.33**	**(0.02, 0.65)***	**0.40**	**(0.08, 0.71)***	0.28	(−0.01, 0.58)	**0.44**	**(0.10, 0.78)***
Elongation factor (%)	0.55	(−4.48, 5.59)	−2.49	(−5.89, 0.91)	−1.84	(−5.27, 1.58)	−1.27	(−4.48, 1.94)	−0.49	(−4.19, 3.21)
Acrosome area of head (%)	−1.77	(−6.26, 2.72)	−0.54	(−3.59, 2.51)	−0.08	(−3.14, 2.98)	−1.10	(−3.97,1.76)	−0.19	(−3.49, 3.12)
Morphology[b]										
Strict criteria (%)	0.41	(−3.83, 4.66)	**−3.33**	**(−6.24, −0.42)***	−1.40	(−4.31, 1.50)	−0.89	(−3.62, 1.83)	−0.97	(−4.11, 2.16)
WHO normal (%)	−0.78	(−13.29, 11.72)	**−9.67**	**(−18.24, −1.09)***	−4.33	(−12.89, 4.23)	−3.35	(−11.36, 4.67)	−3.37	(−12.61, 5.86)
Amorphous (%)	0.62	(−0.37, 1.61)	**0.75**	**(0.07, 1.43)***	0.54	(−0.14, 1.21)	0.46	(−0.18, 1.09)	0.33	(−0.40, 1.06)
Round (%)	−0.17	(−1.10, 0.75)	0.56	(−0.05, 1.18)	0.26	(−0.35, 0.87)	0.36	(−0.21, 0.93)	0.35	(−0.32, 1.02)
Pyriform (%)	0.48	(−0.55, 1.50)	0.52	(−0.17, 1.20)	0.57	(−0.11, 1.26)	0.32	(−0.33, 0.98)	0.41	(−0.33, 1.16)
Bicephalic (%)	0.04	(−0.92, 1.00)	0.30	(−0.36, 0.97)	0.05	(−0.61, 0.72)	0.34	(−0.28, 0.96)	0.40	(−0.32, 1.12)
Taper (%)	0.21	(−0.70, 1.12)	0.13	(−0.49, 0.74)	0.33	(−0.29, 0.95)	0.24	(−0.34, 0.81)	0.33	(−0.33, 0.98)
Megalo head (%)	0.00	(−0.68, 0.68)	**0.72**	**(0.25, 1.20)****	**0.49**	**(0.02, 0.96)***	0.43	(−0.01, 0.87)	**0.54**	**(0.03, 1.05)***
Micro head (%)	0.16	(−0.52, 0.84)	0.19	(−0.27, 0.66)	0.10	(−0.35, 0.56)	0.12	(−0.31, 0.55)	0.16	(−0.33, 0.65)

Continued

Table III *Continued*

Semen parameter	MEHP		MCMHP		MEHHP		MEOHP		MECPP	
	β	(95% CI)	*β*	(95% CI)	*β*	(95% CI)	*β*	(95% CI)	*β*	(95% CI)
Neck or midpiece abnormalities (%)	−0.04	(−0.42, 0.34)	0.23	(−0.03, 0.49)	0.05	(−0.21, 0.31)	0.04	(−0.20, 0.29)	0.00	(−0.28, 0.28)
Coiled tail (%)	−0.09	(−0.54, 0.37)	0.20	(−0.12, 0.51)	0.04	(−0.28, 0.35)	0.00	(−0.30, 0.29)	0.02	(−0.32, 0.35)
Other tail abnormalities (%)	−0.14	(−0.84, 0.56)	−0.26	(−0.74, 0.21)	−0.33	(−0.80, 0.15)	−0.25	(−0.70, 0.19)	−0.27	(−0.79, 0.24)
Cytoplasmic droplet (%)	0.28	(−0.77, 1.34)	**0.89**	**(0.17, 1.61)***	0.34	(−0.38, 1.06)	0.44	(−0.23, 1.12)	0.22	(−0.55, 1.00)
Immature sperm (#)	0.28	(−0.72, 1.28)	**0.90**	**(0.22, 1.58)****	0.59	(−0.09, 1.26)	0.62	(−0.01, 1.26)	0.41	(−0.32, 1.15)

Note: Estimates generated using linear or mixed linear regression models adjusted for age, race, body mass index, income, serum cotinine, urine creatinine, abstinence time, and study site. Statistically significant associations in bold typeface. Analysis for volume, total count, and concentration included $n = 5$ azoospermic men.
DEHP metabolites include MEHP (mono-2-ethylhexyl phthalate), MCMHP (mono-[2-(carboxymethyl) hexyl] phthalate), MEHHP (mono-(2-ethyl-5-hydroxyhexyl) phthalate), MEOHP (mono-(2-ethyl-5-oxohexyl) phthalate), and MECPP (mono-(2-ethyl-5-carboxypentyl) phthalate).
β, beta coefficient; CI, confidence interval; WHO, World Health Organization.
[a]Assessed in two semen samples per man.
[b]Assessed only in the baseline semen sample.
*$P < 0.05$.
**$P < 0.01$.

(−3.62 × 10^6/ml) and MBzP (−3.09 × 10^6/ml) were associated with lower sperm concentration, although the effects were generally weaker than for total sperm count. We detected a counterintuitive association between MCHP and higher semen volume (0.97 ml). We also detected inverse associations for exposure with percent motility, including for MCMHP (−2.01), MEOHP (−1.61), MECPP (−1.88) and MCPP (−2.42). For more refined measures of motility, higher MMP and MCPP concentrations were associated with lower % straightness (−15.30 and −17.28, respectively) and lower % linearity (−11.63 and −11.15, respectively). Higher MEHP was associated with higher average path velocity (11.61 μm/s), curvilinear velocity (20.36 μm/s) and amplitude head displacement (1.72 μm). The impact of ∑DEHPm on semen volume, total sperm count, sperm concentration, and measures of motility echoed results for the individual constituent DEHP metabolites, though with diminished point estimates and fewer significant findings (Supplementary Table SIII).

We assessed several aspects of the morphology of the sperm head and overall in association with higher phthalate concentrations, as shown in Tables III–V. Sperm head size changes included MCMHP and MEHHP with longer length (0.04 μm for each) and perimeter (0.33 and 0.40 μm, respectively), MECPP with larger area (0.72 μm^2) and longer perimeter (0.44 μm), MMP with longer length (0.05 μm), smaller width (−0.15 μm), and smaller elongation factor (−6.16%), MiBP with larger area (0.74 μm^2), and MBzP with larger area (1.03 μm^2) and width (0.16 μm), and with longer perimeter (0.53 μm). We detected multiple associations for sperm morphology parameters as well. MCMHP was associated with a lower proportion of normal sperm using Kruger's strict (−3.33%) and the WHO (−9.67%) criteria, more amorphous (0.75%) and megalo head sperm (0.72%), more sperm with cytoplasmic droplet (0.89%), and more immature sperm (0.90). We also detected a higher proportion of megalo head sperm in association with MEHHP (0.49%) and MECPP (0.54%), and a larger number of immature sperm was further detected in association with higher MMP (0.93) and MEP (0.69). Higher MNP was associated with less normal sperm by strict (−5.74%) and WHO (−19.78%) criteria, with more amorphous sperm (1.23%), and with more cytoplasmic droplet sperm (1.71%). Higher levels of MiBP (1.21%) and MBP (1.23%) were associated with more pyriform sperm. The impact of ∑DEHPm on sperm head morphometry and overall morphology approximated results for the individual constituent DEHP metabolites, though with diminished point estimates (except for sperm head perimeter) and fewer significant findings (Supplementary Table SIII). No associations were indicated for phthalates and our two measures of sperm chromatin stability (data not shown).

Discussion

In this sample of men recruited from the general population upon planning to try for pregnancy, we identified a pattern of diminished semen quality in association with higher concentrations of urinary phthalate monoesters. In particular, higher levels of DEHP metabolites, including MCMHP, MEHHP, MEOHP, and MECPP, as well as MMP, a metabolite of di-methyl phthalate (DMP), and MNP, a metabolite of DNP, were associated with lower motility and with altered sperm head and morphology. Effects for ∑DEHPm were similar to those for individual DEHP metabolites, yet with attenuated impacts and reduced precision, underscoring the importance of assessing individual phthalates. We detected additional, isolated effects for MEP and MCPP, metabolites of DEP and

Table IV Differences in semen quality parameters associated with a one interquartile range increase in natural log-transformed urine di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-octyl phthalate (DOP), and di-isononyl phthalate (DNP) metabolites—LIFE Study (n = 375).

Semen parameter	MMP		MEP		MCPP		MOP		MNP	
	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
General characteristics[a]										
Volume (ml)	0.10	(−0.38, 0.58)	−0.30	(−0.74, 0.14)	−0.06	(−0.60, 0.48)	0.15	(−0.47, 0.78)	−0.64	(−1.44, 0.16)
Viability (%)	−2.23	(−8.94, 4.48)	−2.74	(−8.90, 3.42)	−0.83	(−8.31, 6.65)	4.69	(−4.04, 13.42)	−2.73	(−13.81, 8.35)
Total count ($\times 10^6$)	−2.77	(−5.72, 0.18)	−2.42	(−5.15, 0.30)	−1.70	(−5.01, 1.62)	2.01	(−1.86, 5.89)	**−7.20**	**(−12.11, −2.30)****
Sperm concentration ($\times 10^6$/ml)	**−2.11**	**(−4.11, −0.11)***	−1.01	(−2.86, 0.85)	−1.15	(−3.41, 1.11)	0.91	(−1.73, 3.55)	**−3.62**	**(−6.98, −0.26)***
Sperm motility[a]										
Average path velocity (μm/s)	−7.21	(−15.27, 0.85)	−3.02	(−10.45, 4.42)	−5.99	(−15.00, 3.02)	−0.34	(−10.90, 10.22)	0.11	(−13.29, 13.50)
Straight line velocity (μm/s)	−5.12	(−11.76, 1.53)	−2.06	(−8.17, 4.06)	−7.27	(−14.66, 0.12)	−0.87	(−9.56, 7.81)	−1.39	(−12.40, 9.61)
Curvilinear velocity (μm/s)	−7.10	(−21.22, 7.01)	−5.54	(−18.51, 7.44)	−9.59	(−25.33, 6.15)	2.10	(−16.32, 20.53)	12.78	(−10.58, 36.13)
Amplitude head displacement (μm)	−0.14	(−1.04, 0.75)	−0.31	(−1.13, 0.51)	−0.47	(−1.46, 0.53)	0.50	(−0.67, 1.66)	1.30	(−0.16, 2.76)
Beat cross frequency (Hz)	−2.74	(−7.21, 1.74)	−2.14	(−6.25, 1.97)	−2.19	(−7.17, 2.78)	−1.55	(−7.38, 4.29)	−2.52	(−9.88, 4.84)
Straightness (%)	**−15.30**	**(−27.50, −3.10)***	−6.36	(−17.65, 4.93)	**−17.28**	**(−30.86, −3.70)***	−5.07	(−21.09, 10.96)	−8.50	(−28.74, 11.74)
Linearity (%)	**−11.63**	**(−19.54, −3.73)****	−3.53	(−10.87, 3.81)	**−11.15**	**(−19.97, −2.34)***	−4.73	(−15.13, 5.68)	−11.33	(−24.38, 1.72)
Percent motility (%)	−1.50	(−3.36, 0.35)	−0.83	(−2.54, 0.88)	**−2.42**	**(−4.49, −0.35)***	1.19	(−1.24, 3.61)	−1.13	(−4.23, 1.97)
Sperm head[a]										
Length (μm)	**0.05**	**(0.01, 0.09)***	0.02	(−0.02, 0.06)	0.03	(−0.02, 0.07)	−0.01	(−0.06, 0.04)	0.03	(−0.03, 0.10)
Area (μm^2)	−0.07	(−0.66, 0.52)	0.27	(−0.28, 0.81)	0.36	(−0.30, 1.01)	0.14	(−0.63, 0.91)	−0.10	(−1.08, 0.88)
Width (μm)	**−0.15**	**(−0.27, −0.03)***	0.01	(−0.10, 0.12)	0.04	(−0.09, 0.18)	0.02	(−0.14, 0.18)	−0.12	(−0.32, 0.08)
Perimeter (μm)	0.21	(−0.14, 0.55)	0.18	(−0.14, 0.49)	0.20	(−0.18, 0.58)	−0.03	(−0.48, 0.42)	0.15	(−0.43, 0.72)
Elongation factor (%)	**−6.16**	**(−9.81, −2.52)****	−1.09	(−4.49, 2.31)	−0.72	(−4.87, 3.44)	1.05	(−3.77, 5.88)	−4.66	(−10.83, 1.50)
Acrosome area of head (%)	0.50	(−2.81, 3.81)	0.37	(−2.67, 3.42)	0.49	(−3.22, 4.19)	−2.59	(−6.89, 1.72)	−2.56	(−8.05, 2.93)
Morphology[b]										
Strict criteria (%)	−1.86	(−5.03, 1.31)	−0.55	(−3.41, 2.30)	−1.49	(−4.97, 1.99)	−0.06	(−4.08, 3.96)	**−5.74**	**(−10.94, −0.54)***
WHO normal (%)	−7.06	(−16.38, 2.27)	−1.23	(−9.64, 7.17)	−5.27	(−15.52, 4.99)	−3.90	(−15.74, 7.93)	**−19.78**	**(−35.06, −4.50)***
Amorphous (%)	0.23	(−0.52, 0.97)	−0.12	(−0.79, 0.55)	0.26	(−0.55, 1.08)	0.14	(−0.80, 1.08)	**1.23**	**(0.01, 2.45)***
Round (%)	0.10	(−0.58, 0.78)	0.25	(−0.37, 0.86)	0.06	(−0.71, 0.83)	−0.17	(−0.99, 0.65)	0.23	(−0.87, 1.34)
Pyriform (%)	0.61	(−0.13, 1.36)	0.20	(−0.47, 0.87)	0.17	(−0.65, 0.98)	0.06	(−0.88, 0.99)	0.72	(−0.50, 1.94)
Bicephalic (%)	0.07	(−0.61, 0.75)	0.43	(−0.18, 1.03)	0.15	(−0.62, 0.91)	0.64	(−0.24, 1.52)	0.25	(−0.86, 1.36)
Taper (%)	0.65	(−0.01, 1.30)	0.42	(−0.17, 1.02)	−0.08	(−0.81, 0.65)	0.08	(−0.76, 0.92)	0.69	(−0.39, 1.76)
Megalo head (%)	−0.09	(−0.61, 0.42)	0.05	(−0.41, 0.51)	0.12	(−0.45, 0.69)	−0.04	(−0.69, 0.60)	−0.11	(−0.97, 0.76)

Continued

Table IV *Continued*

Semen parameter	MMP		MEP		MCPP		MOP		MNP	
	β	(95% CI)	*β*	(95% CI)	*β*	(95% CI)	*β*	(95% CI)	*β*	(95% CI)
Micro head (%)	−0.24	(−0.73, 0.25)	0.16	(−0.29, 0.62)	0.18	(−0.37, 0.73)	−0.10	(−0.75, 0.55)	−0.24	(−1.09, 0.61)
Neck or midpiece abnormalities (%)	0.15	(−0.14, 0.43)	−0.05	(−0.31, 0.20)	−0.15	(−0.46, 0.16)	0.20	(−0.16, 0.56)	0.32	(−0.15, 0.79)
Coiled tail (%)	0.18	(−0.16, 0.53)	0.04	(−0.27, 0.35)	0.35	(−0.03, 0.72)	0.12	(−0.31, 0.56)	0.10	(−0.47, 0.66)
Other tail abnormalities (%)	0.00	(−0.52, 0.53)	−0.03	(−0.50, 0.44)	−0.43	(−1.00, 0.15)	0.31	(−0.35, 0.96)	0.41	(−0.44, 1.27)
Cytoplasmic droplet (%)	0.32	(−0.46, 1.11)	−0.16	(−0.87, 0.55)	−0.28	(−1.14, 0.59)	−0.11	(−1.11, 0.88)	**1.71**	**(0.42, 3.00)****
Immature sperm (#)	**0.93**	**(0.19, 1.66)***	**0.69**	**(0.01, 1.36)***	0.33	(−0.48, 1.15)	0.08	(−0.83, 0.99)	0.45	(−0.74, 1.65)

Note: Estimates generated using linear or mixed linear regression models adjusted for age, race, body mass index, income, serum cotinine, urine creatinine, abstinence time, and study site. Statistically significant associations in bold typeface. Analysis for volume, total count, and concentration included $n = 5$ azoospermic men.
DMP metabolites include MMP (mono-methyl phthalate); DEP metabolites include MEP (mono-ethyl phthalate); DOP metabolites include MCPP (mono-(3-carboxypropyl) phthalate) and MOP (mono-n-octyl phthalate); DNP metabolites include MNP (mono-isononyl phthalate).
β, beta coefficient; CI, confidence interval; WHO, World Health Organization.
[a]Assessed in two semen samples per man.
[b]Assessed only in the baseline semen sample.
*$P < 0.05$.
**$P < 0.01$.

di-octyl phthalate (DOP), respectively. MEHP was unexpectedly associated with higher sperm motility in our study, and we found no associations between MBP and motility or for sperm chromatin. Still, given the number of statistical tests, we the possibility of chance findings is important to acknowledge.

Associations of urinary phthalates with human semen quality have been assessed most commonly in clinical populations, recruiting male partners of couples undergoing infertility treatment (Hauser *et al.*, 2006; Wirth *et al.*, 2008; Liu *et al.*, 2012; Toshima *et al.*, 2012; Jurewicz *et al.*, 2013). In studies of clinical populations, higher levels of phthalates including MBP have been observed to be related to lower sperm motility (Hauser *et al.*, 2006; Jurewicz *et al.*, 2013) and lower sperm concentration (Hauser *et al.*, 2006; Liu *et al.*, 2012). Measures of DNA damage, including comet extent, tail-distributed moment, and % fragmentation, have also been seen with higher urinary MEP, MEHHP, and adjusted MEHP (Hauser *et al.*, 2007). Despite detecting numerous functional changes, we did not observe associations of phthalate metabolites with measures of DNA damage, which might be related to misclassification if *in utero* exposure at the time of testes development is important (Sharpe, 2006), or possibly is a consequence of the lower prevalence of measures of this outcome in a general population.

To the best of our knowledge, this study is the most comprehensive assessment of phthalates and semen quality in a non-clinical population conducted to date. The findings of lower sperm concentration, diminished motility, and a higher proportion of abnormal morphology are consistent with those from some clinical populations that report adverse associations between phthalates and semen quality (Kay *et al.*, 2014). Only three prior studies have described the impact of urine phthalates on semen quality among men recruited from general, non-clinical populations. A Swedish study of 234 young military conscripts (Jönsson *et al.*, 2005) reported 8.8% lower sperm motility in association with higher urine MEP at median levels higher than ours (240 ng/ml), and no associations for measured MEHP (<15 ng/ml), MBP (78 ng/ml), or MBzP (16 ng/ml), or for their assessment of sperm concentration and chromatin integrity. In a large study of young Danish men ($n = 881$), no consistent associations were detected for 14 urinary phthalate monoesters or their sums and semen volume or sperm concentration, count, motility, or morphology (Joensen *et al.*, 2012). The median levels of MEHP (4.0 ng/ml), MEHHP (23 ng/ml), MEOHP (14 ng/ml), MCPP (5.0 ng/ml), MOP (0.1 ng/ml), MNP (0.6 ng/ml), MiBP (58 ng/ml), MBP (28 ng/ml), and MBzP (34 ng/ml) in that study were higher or similar to values in our study, while median levels of MECPP (15 ng/ml) and MEP (78 ng/ml) were lower than ours. More recently, no associations were reported for semen volume, for sperm concentration, motility, or morphology, or for sperm chromatin measures, with urine phthalates in 232 Chinese men (Han *et al.*, 2014). Whereas the median MEHP level in that study (1.10 ng/ml) was similar to ours, their median MEP level (3.10 ng/ml) was lower and their median MBP level (18.72 ng/ml) was higher than our measurements.

The three previous studies of urinary phthalates and semen quality in general population samples assessed a limited panel of quality parameters; thus, modest effects may have been missed (Jönsson *et al.*, 2005; Joensen *et al.*, 2012; Han *et al.*, 2014). While the Danish study measured a comprehensive panel of primary and secondary urinary phthalate metabolites, the Swedish and Chinese studies captured exposure to only four primary, hydrolytic phthalate monoesters. Because of the large number of metabolic products of phthalate diesters, and variation in

Table V Differences in semen quality parameters associated with a one interquartile range increase in natural log-transformed urine di-butyl phthalate (DBP), dicyclohexyl phthalate (DCHP), and butylbenzyl phthalate (BzBP) metabolites—LIFE Study ($n = 375$).

Semen parameter	MiBP		MBP		MCHP		MBzP	
	β	(95% CI)	β	(95% CI)	β	(95% CI)	β	(95% CI)
General characteristics[a]								
Volume (ml)	0.08	(−0.48, 0.65)	0.09	(−0.64, 0.81)	**0.97**	**(0.16, 1.77)***	−0.23	(−0.81, 0.35)
Viability (%)	−3.76	(−11.62, 4.11)	−4.40	(−14.48, 5.68)	2.66	(−8.72, 14.04)	−7.49	(−15.57, 0.59)
Total count ($\times 10^6$)	0.88	(−2.59, 4.36)	−0.81	(−5.30, 3.68)	−1.31	(−6.33, 3.70)	**−4.96**	**(−8.53, −1.40)****
Sperm concentration ($\times 10^6$/ml)	0.46	(−1.90, 2.83)	−0.95	(−4.00, 2.10)	−3.01	(−6.40, 0.38)	**−3.09**	**(−5.52, −0.66)***
Sperm motility[a]								
Average path velocity (μm/s)	−3.72	(−13.22, 5.78)	−3.37	(−15.55, 8.81)	7.89	(−5.83, 21.61)	−7.02	(−16.79, 2.76)
Straight line velocity (μm/s)	−1.92	(−9.73, 5.90)	−0.72	(−10.73, 9.3)	7.27	(−4.02, 18.56)	−3.57	(−11.63, 4.48)
Curvilinear velocity (μm/s)	−5.80	(−22.38, 10.78)	−4.88	(−26.15, 16.39)	16.21	(−7.70, 40.12)	−11.93	(−29.00, 5.14)
Amplitude head displacement (μm)	−0.18	(−1.23, 0.87)	−0.41	(−1.75, 0.93)	1.17	(−0.35, 2.69)	−0.51	(−1.59, 0.56)
Beat cross frequency (Hz)	−2.18	(−7.44, 3.08)	0.99	(−5.73, 7.72)	−2.40	(−10.05, 5.25)	−0.30	(−5.72, 5.13)
Straightness (%)	−13.40	(−27.79, 0.98)	−7.28	(−25.75, 11.19)	6.58	(−14.37, 27.53)	−7.78	(−22.65, 7.09)
Linearity (%)	−8.98	(−18.33, 0.38)	−6.51	(−18.49, 5.46)	2.68	(−11.00, 16.36)	−5.50	(−15.16, 4.16)
Percent motility (%)	−0.98	(−3.16, 1.20)	−1.22	(−4.03, 1.58)	0.73	(−2.42, 3.87)	−1.67	(−3.92, 0.58)
Sperm head[a]								
Length (μm)	0.03	(−0.02, 0.08)	0.03	(−0.03, 0.09)	0.01	(−0.06, 0.08)	0.03	(−0.01, 0.08)
Area (μm^2)	**0.74**	**(0.05, 1.43)***	0.59	(−0.30, 1.48)	0.16	(−0.84, 1.16)	**1.03**	**(0.33, 1.74)****
Width (μm)	0.08	(−0.06, 0.22)	0.04	(−0.14, 0.22)	0.01	(−0.20, 0.21)	**0.16**	**(0.01, 0.31)***
Perimeter (μm)	0.40	(0.00, 0.80)	0.36	(−0.16, 0.88)	0.12	(−0.46, 0.70)	**0.53**	**(0.12, 0.94)***
Elongation factor (%)	−0.18	(−4.52, 4.15)	−0.97	(−6.56, 4.62)	−0.99	(−7.19, 5.20)	1.01	(−3.47, 5.49)
Acrosome area of head (%)	2.30	(−1.57, 6.16)	3.73	(−1.24, 8.70)	0.35	(−5.22, 5.92)	2.48	(−1.51, 6.48)
Morphology[b]								
Strict criteria (%)	−1.73	(−5.37, 1.91)	−2.60	(−7.33, 2.12)	−0.43	(−5.57, 4.71)	−2.04	(−5.86, 1.78)
WHO normal (%)	−4.43	(−15.15, 6.29)	−7.03	(−20.95, 6.89)	−2.62	(−17.76, 12.52)	−5.71	(−16.97, 5.56)
Amorphous (%)	−0.06	(−0.91, 0.79)	−0.33	(−1.44, 0.77)	−0.37	(−1.57, 0.83)	−0.24	(−1.13, 0.65)
Round (%)	−0.02	(−0.84, 0.81)	−0.15	(−1.14, 0.83)	−0.35	(−1.48, 0.79)	−0.19	(−1.02, 0.63)
Pyriform (%)	**1.21**	**(0.36, 2.05)****	**1.23**	**(0.13, 2.33)***	0.60	(−0.59, 1.79)	0.66	(−0.24, 1.56)
Bicephalic (%)	0.23	(−0.59, 1.06)	0.34	(−0.79, 1.46)	0.39	(−0.60, 1.38)	0.47	(−0.31, 1.26)
Taper (%)	−0.26	(−1.03, 0.51)	−0.42	(−1.42, 0.57)	0.07	(−1.02, 1.17)	0.03	(−0.77, 0.82)
Megalo head (%)	0.08	(−0.50, 0.66)	0.06	(−0.70, 0.81)	0.61	(−0.20, 1.43)	0.19	(−0.43, 0.81)
Micro head (%)	0.07	(−0.51, 0.65)	0.18	(−0.57, 0.92)	−0.30	(−1.09, 0.49)	0.43	(−0.15, 1.01)
Neck or midpiece abnormalities (%)	−0.04	(−0.36, 0.29)	0.08	(−0.35, 0.50)	0.36	(−0.10, 0.81)	−0.01	(−0.35, 0.33)
Coiled tail (%)	0.02	(−0.37, 0.41)	0.20	(−0.31, 0.71)	−0.02	(−0.58, 0.53)	−0.18	(−0.59, 0.23)
Other tail abnormalities (%)	−0.17	(−0.76, 0.43)	−0.20	(−0.97, 0.58)	0.06	(−0.78, 0.90)	0.48	(−0.15, 1.10)
Cytoplasmic droplet (%)	0.70	(−0.20, 1.60)	0.91	(−0.26, 2.08)	1.22	(−0.05, 2.49)	0.61	(−0.34, 1.56)
Immature sperm (#)	0.21	(−0.66, 1.09)	0.57	(−0.57, 1.71)	0.75	(−0.47, 1.98)	0.44	(−0.47, 1.35)

Note: Estimates generated using linear or mixed linear regression models adjusted for age, race, body mass index, income, serum cotinine, urine creatinine, abstinence time, and study site. Statistically significant associations in bold typeface. Analysis for volume, total count, and concentration included $n = 5$ azoospermic men.

DBP metabolites include MiBP (mono-isobutyl phthalate) and MBP (mono-n-butyl phthalate); DCHP metabolites include MCHP (monocyclohexyl phthalate); BzBP metabolites include MBzP (mono-benzyl phthalate) and MBP.

β, beta coefficient; CI, confidence interval; WHO, World Health Organization.

[a]Assessed in two semen samples per man.

[b]Assessed only in the baseline semen sample.

*$P < 0.05$.

**$P < 0.01$.

Table VI Summary of significant effects between urinary phthalate monoesters (ng/ml) and semen quality parameters—LIFE Study ($n = 375$).

	MEHP	MCMHP	MEHHP	MEOHP	MECPP	MMP	MEP	MCPP	MNP	MiBP	MBP	MCHP	MBzP
General characteristics[a]													
Volume (ml)	–	–	–	–	–	–	–	–	–	–	–	↑	–
Total count ($\times 10^6$)	–	↓	↓	–	–	–	–	–	↓	–	–	–	↓
Sperm concentration ($\times 10^6$/ml)	–	↓	↓	↓	–	↓	–	–	↓	–	–	–	↓
Sperm motility[a]													
Average path velocity (μm/s)	↑	–	–	–	–	–	–	–	–	–	–	–	–
Curvilinear velocity (μm/s)	↑	–	–	–	–	–	–	–	–	–	–	–	–
Amplitude head displacement (μm)	↑	–	–	–	–	–	–	–	–	–	–	–	–
Straightness (%)	–	–	–	–	–	↓	–	↓	–	–	–	–	–
Linearity (%)	–	–	–	–	–	↓	–	↓	–	–	–	–	–
Percent motility (%)	–	↓	–	↓	↓	–	–	↓	–	–	–	–	–
Sperm head[a]													
Length (μm)	–	↑	↑	–	–	↑	–	–	–	–	–	–	–
Area (μm^2)	–	–	–	–	↑	–	–	–	–	↑	–	–	↑
Width (μm)	–	–	–	–	–	↓	–	–	–	–	–	–	↑
Perimeter (μm)	–	↑	↑	–	↑	–	–	–	–	–	–	–	↑
Elongation factor (%)	–	–	–	–	–	↓	–	–	–	–	–	–	–
Morphology[b]													
Strict criteria (%)	–	↓	–	–	–	–	–	–	↓	–	–	–	–
WHO normal (%)	–	↓	–	–	–	–	–	–	↓	–	–	–	–
Amorphous (%)	–	↑	–	–	–	–	–	–	↑	–	–	–	–
Pyriform (%)	–	–	–	–	–	–	–	–	–	↑	↑	–	–
Megalo head (%)	–	↑	↑	–	↑	–	–	–	–	–	–	–	–
Micro head (%)	–	–	–	–	–	–	–	–	–	–	–	–	–
Cytoplasmic droplet (%)	–	↑	–	–	–	–	–	–	↑	–	–	–	–
Immature sperm (#)	–	↑	–	–	–	↑	↑	–	–	–	–	–	–

Note: Significant effects detected for 14 phthalate monoesters with 34 semen parameters (no effects detected for MOP or for viability (%), straight line velocity (μm/s), beat cross frequency (Hz), acrosome area of head (%), round sperm (%), bicephalic sperm (%), taper sperm (%), neck or midpiece abnormality (%), coiled tail (%), or other tail abnormalities (%). Effects generated using linear or mixed linear regression models adjusted for age, race, body mass index, income, serum cotinine, urine creatinine, abstinence time, and study site. Analysis for volume, total count, and concentration included $n = 5$ azoospermic men.

MEHP, mono-2-ethylhexyl phthalate; MCMHP, mono-[2-(carboxymethyl) hexyl] phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MMP, mono-methyl phthalate; MEP, mono-ethyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MOP, mono-n-octyl phthalate; MNP, mono-isononyl phthalate; MiBP, mono-isobutyl phthalate; MBP, mono-n-butyl phthalate; MCHP, monocyclohexyl phthalate; MBzP, mono-benzyl phthalate; WHO, World Health Organization.

[a]Assessed in two semen samples per man.

[b]Assessed only in the baseline semen sample.

–, no significant effect; ↑, significant increase ($P < 0.05$); ↓, significant decrease ($P < 0.05$).

the half-lives of phthalate monoesters, measurement of a large panel of monoesters may be better able to capture phthalate exposure than is assessment of only primary metabolites (Koch and Calafat, 2009). Our comprehensive analysis of 14 primary and secondary phthalate monoester metabolites as predictors of 34 semen quality indicators in a general population offers improved insight into subtle associations unavailable to prior investigators.

A growing number of publications report oxidative stress (OS) induced damage to male reproductive organs in association with phthalates exposure, and spermatozoa with high concentrations of polyunsaturated fatty acids appear to be especially vulnerable to OS-induced lipid peroxidation (Lenzi *et al.*, 2000). Even at low, environmentally relevant doses, MBP and MEHP elicit destruction of Sertoli tight cell junctions *in vitro* (Zhang *et al.*, 2008). Higher OS and OS-associated DNA damage has also been reported by epidemiologic studies of phthalate exposures (Kadiiska *et al.*, 2005; Hong *et al.*, 2009). In an earlier LIFE study, urine 8-hydroxy-2′-deoxyguanosine levels were positively correlated to urine MEHP ($r = 0.59$, $P = 0.000$) and MiBP ($r = 0.10$; $P = 0.033$) in men (Guo *et al.*, 2014). In the current analysis, we describe previously unreported associations for urine MCMHP, MEHHP, MECPP, MMP, MiBP, and MBzP with sperm head parameters, possibly indicative of disrupted spermiogenesis, the transformation of spherical spermatids into elongated spermatozoa (Hess and de Franca, 2008). Still, mechanisms by which phthalates may impact semen quality in humans remain unclear.

Our work has several important limitations including phthalate levels measured using a single baseline urine specimen for exposure assessment. Misclassification is a concern related to the short *in vivo* half-lives of phthalates (Anderson *et al.*, 2001). Nevertheless, in assessment of temporal stability of phthalate metabolite measures, a single spot urine was moderately predictive of overall three month averages in men (Hauser *et al.*, 2004), corresponding to the cycle of human sperm production and maturation (Hess and de Franca, 2008). Additional studies have reached similar conclusions for women (Hoppin *et al.*, 2002), although indicating lower reliability for DEHP metabolites (Peck *et al.*, 2010). Misclassification related to this kind of temporal variability represents a bias that would tend to be toward the null hypothesis. Longer term temporal trends have been observed related to US industry substitutions for DEHP, DBP, and BBzP (Zota *et al.*, 2014), and may help to explain the low exposure levels observed in LIFE study participants. In fact the levels in our study were generally lower than those reported for US men overall (CDC, 2015). Temporal trends, along with geographic and population-specific differences, would not impact our findings, but likely contribute to the different results across studies. Also of note, we used a next day semen analysis approach, given the logistical challenges associated with our study conducted outside of a clinical setting. This strategy has been successful in prior studies (Royster *et al.*, 2000), yielding reliable information for most semen parameters of interest (Stovall *et al.*, 1994; Morris *et al.*, 2003). Although some sperm survive past 24 h (Stovall *et al.*, 1994) and refrigerated samples maintain sperm chromatin structure (Morris *et al.*, 2003), our next day motility end-points need to be cautiously interpreted, given the absence of established validity for interpreting findings as with clinical semen analysis.

Phthalate metabolites may impact male obesity (Hatch *et al.*, 2008); associations among overweight and obese men might differ from associations in normal weight men. Men in our sample were skewed toward higher BMI (83% overweight/obese), not dissimilar to the high proportion (69%) reported for US men overall (National Center for Health Statistics, 2015). Still, higher phthalate levels tend to be associated with higher BMI, and so we suggest caution in generalizing our results to men with normal BMI.

We conducted a large number of statistical tests, consistent with our focus on individual phthalate monoesters and detection of signals for associations with a comprehensive panel of semen quality parameters. We fully explored our data to assess previously reported associations and to identify signals to guide new research directions (Goldberg and Silbergeld, 2011). Taken together, these findings suggest a general pattern of lower motility, larger sperm head, and more abnormal forms in association with higher urine phthalates, lending credibility to our analysis. Still, an inflated type-1 error rate might manifest as false-positive findings, and significant associations reported herein may result from chance.

In conclusion, our results suggest that phthalate diesters may negatively impact semen quality, even at low exposure levels in the general population. These associations are consistent with our earlier report of diminished couple-level fecundability in association with higher levels of male urine phthalate monoesters (Buck Louis *et al.*, 2014). Given the widespread nature of phthalates, even subtle effects on semen quality would have substantial implications at the population level for public health, as well as increased health care costs; a recent European Union sanctioned report suggests phthalates may represent a cost of €4.71 billion in annual assisted reproductive technology expenses (Hauser *et al.*, 2015). Further research clarifying phthalate effects on reproductive health and to establish thresholds for adverse reproductive effects is merited given the magnitude of the potential public health impact.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

M.S.B. interpreted the data, drafted and revised the manuscript for critically important content, and approved the final version to be submitted; B.W.W. interpreted the data, drafted and revised the manuscript for critically important content, and approved the final version to be submitted; Z.C. completed the data analysis, revised the manuscript for critically important content, and approved the final version to be submitted; A.Y. completed the data analysis, revised the manuscript for critically important content, and approved the final version to be submitted; K.K. completed the laboratory analysis, revised the manuscript for critically important content, and approved the final version to be submitted; G.M.B.L. designed the LIFE Study, revised the manuscript for critically important content, and approved the final version to be submitted.

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Conflict of interest

The authors declare they have no actual or potential competing financial interests.

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